(54) Title: IMMOBILIZED POLYETHYLENE OXIDE STAR MOLECULES FOR BIOAPPLICATIONS

(57) Abstract

This invention pertains to a method for immobilizing polyethylene oxide (PEO) star molecules in the form of hydrogel layers. The PEO star molecules are biocompatible and demonstrate non-thrombogenic properties. As such, the PEO star molecule layers have numerous biomedical applications, such as on contact lenses. The hydrogel layers contain a high percentage of terminal hydroxyl groups for attachment of affinity ligands and can be used for separating and purifying therapeutic proteins.

IMMOBILIZED POLYETHYLENE OXIDE STAR
MOLECULES FOR BIOAPPLICATIONS

Background of the Invention

Polyethylene oxide (PEO) is an important
biomaterial because it is non-adsorptive toward
biopolymers, and is non-thrombogenic, i.e, it does not
adsorb proteins of the intrinsic clotting system nor of
the platelet membrane. However, when PEO is combined
with other molecules at the surface, thrombogenicity
may be enhanced. Okkema, J. Biomat. Sci. 1:43-62
(1989). Thus, it is essential that no other molecular
entity besides PEO be accessible to proteins. It has
been widely studied as a blood-contacting biomaterial
in various forms: in segmented polyurethanes, in block
copolymers with styrene or siloxane blocks, end-linked
into junctions through isocyanate reactions, as side-
chains on acrylate polymers and as hydrogels cross-
linked from PEO solutions.

PEO is naturally soluble in water and certain
organic solvents. Therefore, in order to render PEO
insoluble it must be cross-linked, or end-linked to a
support. The manner in which this is accomplished
often affects physical and chemical properties of PEO.

Chemical cross-linking of PEO can be employed, but
the chemical cross-linking agent (e.g., a
polyfunctional isocyanate) may be incorporated into the

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PEO. If exposed at the ultimate surface, such a
chemical moiety can cause adverse biopolymer reactions,
including non-specific binding of proteins and platelet
adhesion.

5   Physically cross-linked PEO produced from
polyethylene oxide-polystyrene multiblock polymers or
from polyether-urethanes suffers from the presence of
the non-PEO materials at the surface. Adverse
biological reactions caused by the non-PEO material may
10   often be avoided if the molecular weight of the PEO is
made higher than about 5,000. However, such material
tends to swell excessively in water and is fragile.

        End-linking PEO to supports by various means, so
as to leave an available hydroxyl groups for attachment
15   of an affinity ligand, for example, is not easily
carried out if the molecular weight of the PEO is more
than about 1,000. Furthermore, complete coverage of a
surface by end-linking PEO is very difficult, unless
the molecular weight is relatively high (several
20   thousand).

        Various forms of PEO have also been widely used as
a molecular leash for affinity ligands and enzymes.
Golander et al., Int. Chem. Congress of Pacific Basin
Societies, Abstract No. 253, Honolulu, Hawaii, December
25   17-22, 1989, Harris, J. Macromolecular Sci. C25:325-373
(1985); Holmberg, Int. Chem. Congress of Pacific Basin
Societies, Abstract No. 255, Honolulu, Hawaii, December
17-22, 1989. Typically, PEO has terminal hydroxyl
groups which can be activated for attachment to
30   biopolymers. Most processes for forming PEO
biomaterials, however, reduce the hydroxyl content to

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very low values or zero. In order to produce a cross-
linked PEO having a significant concentration of
terminal hydroxyls, low molecular weight PEO (2,000 to
10,000) are required but often result in fragile
5   materials. Alternatively, using short PEO side chains
on macromonomers like polyethylene glycol methacrylate
may result in exposure of the methacrylate residues at
the surface, and these short PEO side chains are almost
invariably methyl terminated, so that no hydroxyl
10   exists for subsequent attachment of ligands.
       Thus, a need exists for a method of immobilizing
PEO to a support surface without detracting from its
physical properties and biological compatibility. In
addition, it would be desirable to provide a material
15   having a high concentration of hydroxyl groups for
attachment to biopolymers.

Summary of the Invention
       This invention pertains to a method for covalently
immobilizing polyethylene oxide star molecules onto a
20   support surface and to layers produced by the method.
The PEO star molecules are immobilized on the support
surface in the form of layers using ionizing radiation,
or hydroxyl group activation followed by chemical
coupling. The resulting PEO layers have a high
25   concentration of terminal hydroxyl groups which are
available for attachment to biospecific affinity
ligands. Thus fitted with ligands, the immobilized PEO
star molecules can be used as a tool for separating and
purifying biological molecules, while greatly reducing
30   or eliminating non-specific binding.

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The PEO star molecule layers also have non-
thrombogenic properties which make them suitable for
applications in which blood contact is required. They
are highly biocompatible and have excellent mechanical
5   durability for numerous biomedical applications,
including intravenous catheters and implantable
vascular prostheses. The layers of this invention can
be formed on contact lens materials providing highly
hydrophilic protein-resistant surfaces.

10   Brief Description of the Drawings
Figure 1a shows a Type I PEO star molecule having
a divinyl benzene (DVB) core and PEO chains attached
thereto.
Figure 1b shows a Type II PEO star molecule having
15   a DVB core and PEO chains attached thereto by
polystyrene (PS) chains.
Figure 2 shows overlapping PEO star molecules
(Type I) which are cross-linked to each other by
electron irradiation.
20   Figure 3 shows several PEO star molecules (Type I)
covalently attached to a support surface by tresylated
hydroxyl groups.
Figure 4 illustrates the attachment of a
biopolymer (IgG) to the surface of immobilized PEO star
25   molecules.

Detailed Description of the Invention
Polyethylene oxide star macromolecules have been
previously described by Lutz et al., Makromol. Chemie
189:1051 (1988) and Gnanou et al., Makromol. Chemie

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189:2893-97 (1988), the teachings of which are
incorporated by reference herein. The star molecules
are synthesized by anionic polymerization from divinyl
benzene (DVB), ethylene oxide and optionally styrene.
5   They have a core of divinyl benzene (typically on the
order of about 50 angstroms) from which a predetermined
number of polyethylene oxide chains or "arms" are
grown. Generally, the DVB core represents about two
weight percent or less of the total star molecule
10 weight. The cores however can be of polymeric material
other than divinyl benzene. The length of each PEO
chain or arm corresponds to molecular weight and
typically ranges from about 1,000 to about 10,000
atomic mass units (a.m.u.). Preferably, each star
15 molecule will have from about six to about 200 arms.
The total molecular weight of the star, which is
approximately the product of the molecular weight of
each arm and the total number of arms, ranges from
about 10^5 to about 10^6 a.m.u. Two variations of PEO
20 star molecules are shown in Figures 1A and 1B and are
described herein as Type I and Type II, respectively.
Type I star molecules contain a plurality of hydroxy-
terminated PEO chains (hydrophilic) that are attached
to a hydrophobic DVB core by non-hydrolyzable carbon-
25 carbon bonds. Type II PEO star molecules are of
similar composition except that the PEO chains are
attached to the DVB core via hydrophobic polystyrene
(PS) chains.

The concentration of hydroxy-termini on the PEO
30 arms can be determined in advance by selection of the
gross concentration of star molecules and the number of

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arms carried by the molecule. For example, a star
molecule of 100,000 molecular weight with twenty PEO
arms has twenty hydroxyl groups. To obtain comparable
hydroxyl concentrations with linear PEO polymers, the
5   molecular weight would have to be lowered to 10,000.
     The PEO star molecules can be immobilized on a
support surface of any geometry (e.g., particles,
porous plastic cores, thin plastic film, biomedical
device, contact lenses) using ionizing radiation.
10   According to the method, PEO star molecules are
     dissolved or suspended in an aqueous solution
     (preferably water) in a concentration sufficient to
     provide enough star molecules to cover the support
     surface to a desired thickness. Typically, a
15   sufficient concentration will be around 5 to 15
     wt/vol%. Type I star molecules form optically clear
     homogeneous solutions in water, while Type II star
     molecules form faintly turbid to opaque suspensions,
     due to the presence of polystyrene. The resulting
20   solution is then deposited onto the support surface,
     such as by spraying, spreading, rotating the support or
     centrifugation.
     The star molecules in solution are then cross-
     linked together and to the surface by exposing them to
25   electron beam radiation which creates free radicals on
     the arms at random positions, and on the surface.
     Random coupling then results in the formation of a
     layer. If the solvent is water, the resulting layer is
     a hydrogel layer. The term "hydrogel" refers to a
30   broad class of polymeric materials which are swollen
     extensively in water but which do not dissolve in

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water. Typically, the solution is exposed to electron
radiation in the range of between about one to about
ten megarads, most preferably four megarads. Gamma
radiation can be used as the radiation source but may
5   result in the degradation of the star molecules unless
oxygen is scrupulously excluded. Cross-linking via
free radical coupling occurs randomly between segments
of the PEO arms, thus allowing the terminal hydroxyl
groups to remain available for subsequent activation,
10   such as for coupling affinity ligands to the PEO arms.
        Figure 2 shows several Type I PEO star molecules
cross-linked together by electron radiation. The
resulting hydrogel layers are of variable thickness but
are typically on the order of magnitude of greater than
15   1μM. The thickness of the hydrogel layer can be
regulated by various techniques, such as doctor-blade
spreading on a support web or centrifugal casting in
tubes.
        An advantage of electron radiation cross-linking
20   is that the cross-linking reaction proceeds very
rapidly, at a rate of approximately one foot per second
in the case of web coating. Under a nitrogen blanket,
the reaction proceeds by free-radical coupling to
produce an essentially pure product without uptake of
25   oxygen. As such, the cross-linking reaction does not
alter the chemical composition of the star molecules.
Other known cross-linking techniques tend to introduce
chemical components which may subsequently affect its
biocompatibility. Further, the hydrogel layer presents
30   a surface for contacting biological materials (e.g.,
blood) which is essentially hydrated PEO chains. As

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such, the DVB and PS components are inaccessible or not recognizable to these biological molecules.

The resulting hydrogels have significantly greater mechanical strength than hydrogels formed from ordinary
5   linear PEO having the same range of molecular weight as the star (i.e., 100,000 to 300,000). A gel made from 10 wt.% of 100,000 molecular weight linear PEO under identical dosage would have two to ten times lower tensile strength than the network formed from star
10   molecules, and would have only one tenth the number of hydroxyl groups per unit area of surface. The concentrations of hydroxyl ends obtained by stars would translate to linear polyethylene oxide of about 5,000 a.m.u. or less. Such low molecular weight polymers
15   cannot be cross-linked at all, or form gels of low strength with considerable soluble fraction.

In another embodiment, the star molecules can be covalently immobilized to a support surface by tresylation of the terminal hydroxyl groups. The
20   support surface and star molecules are each pretreated prior to immobilization. As such, the support surface should contain active functional groups for immobilizing tresylated star molecules thereto, such as amino and/or thiol groups. Likewise, the star
25   molecules should be tresylated in an appropriate solvent prior to contacting with the support surface. Tresylation is particularly convenient since the PEO is solvated by media appropriate to tresyl chloride (e.g., dichloromethane, chloroform). This method results in a
30   monolayer coating of the hydrogel over the support surface.

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According to this method, an organic solvent, such
as dichloromethane, comprising PEO star molecules is
exposed to tresyl chloride, under conditions to affix
the tresyl groups to hydroxy-termini on the star
5   molecules. The resulting tresylated PEO star molecules
are then precipitated and recovered, ultimately as a
dry active product. Just prior to use, the tresylated
PEO star molecules are dissolved in an aqueous solution
at a pH of ten or above, so as to favor reaction with
10   amino and/or thiol groups already present on the
support surface. The pH-adjusted solution is contacted
with a support surface that contains amino and/or thiol
groups, under conditions whereby the star molecules
become covalently bound in a dense layer to the support
15   surface.

This process is further described below by way of
illustration. For example, a Cellophane™ (cellulose
containing plasticizers) containing support is placed
in a bath of tetrahydrofuran and tresyl chloride. The
20   hydroxyl groups on the surface of the Cellophane™
support are then tresylated. Once tresylated, the
Cellophane™ support is aminated in a water solution of
ethylene diamine (pH 10) which results in binding the
group $-HN-CH_2CH_2NH_2$ to the activated hydroxyl groups.
25   Unreacted diamine is removed by washing with
tetrahydrofuran. Likewise, star molecules are
tresylated, purified, and then placed into an aqueous
buffer (pH 10) containing the aminated Cellophane™
support. After a period of approximately one hour, the
30   Cellophane™ support is removed from the solution and
rinsed to wash off any unbound star molecules. The

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star molecules become bound to the amino group via the
tresylated hydroxyls. Figure 3 shows several PEO star
molecules immobilized on a support surface. The
attachment results from the reaction of amino groups on
5   the support surface with tresylated hydroxyls on the
star molecules.

In addition to tresyl chloride, other reagents
can be used to react with the terminal hydroxyl groups
on the PEO chains. These reagents include tosyl
10 chloride (p-toluene sulfonyl chloride), mesyl chloride
(methane sulfonyl chloride), epichlorhydrin, cyanuric
chloride ($C_3N_3Cl_3$), carbonyl diimidazole (CDI) and a
mixture of succinic anhydride and succinimide. These
reactions are generally described by Harris,
15 "Laboratory Synthesis of Polyethylene Glycol
Derivatives," J. Macromolecular Sci. Reviews in Macro.
Chem. Phys., C25(3), 325-373 (1985). For example, the
hydroxy-terminated polyethylene oxide is reacted with
tosyl chloride or mesyl chloride to form a tosylated or
20 mesylated star molecule, respectively. In each case
the activated PEO arms can be reacted with any molecule
containing an amino or thiol group. By-products
liberated by the reaction of the amino or thiol
containing compound with the activated PEO arms include
25 tresyl, mesyl or tosyl sulfonic acid, HCl (reaction of
cyanuric chloride), imidazole (reaction of CDI), or
N-hydroxylsuccinimide (reaction of succinic anhydride
and succinimide). There is no by-product liberated in
the epichlorhydrin reaction. Among the surface
30 activation chemistries described above, tresylation is
the most efficient method in terms of speed and product

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recovery. Any molecule containing an amino or thiol
group, for example a protein, can now become covalently
attached to a tresylated, tosylated, or mesylated
polyethylene oxide chain by the formation of the very
5   stable -NC- or -SC- bond with the elimination of the
respective sulfonic acid: tresyl, tosyl or mesyl.
    The PEO star molecules can be covalently bonded
onto an appropriate support surface using the methods
previously described to thereby protect or "mask" the
10   support from recognition by biopolymers. A dense
monolayer coating of PEO star molecules can be
accomplished by allowing contact between a solution or
above pH 10 of activated (e.g. by tresyl) PEO star
molecules and a surface containing, for example, amino
15   or thiol groups in close proximity, such that the star
molecules pack closely together, each attached by one
or more arms to the surface at the site of the amino or
thiol group. The remaining arms are available for
attaching biopolymers or affinity ligands. The PEO-
20   coated support surface can then be exposed to a
biopolymer having amino or thiol groups which can
couple to available tresylated hydroxyl groups. These
available groups function as molecular leashes or
tethers for the biopolymer. For example, anti-Protein
25   C antibody can be attached to the star molecules and
will be selective for its antigen, Protein C. The PEO
monolayer prevents adsorption of the biopolymers onto
the support surface and can thereby reduce or eliminate
non-specific binding of undesired biopolymers. Figure
30   4 demonstrates the use of star molecules for attaching
affinity ligands, such as Immunoglobulin G. The symbol

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♦ represents a covalent linkage between a PEO arm and
an amino group on the support; ● represents a covalent
linkage between a PEO arm and an amino or thiol group
on IgG; ★ represents an endcapped previously tresylated
5 hydroxyl (e.g., by treatment with mercaptoethanol).

A support surface can be aminated by ammonia
plasma. The pH of a ten percent tresylated PEO star
solution is adjusted to about ten or greater. The
solution is then contacted with the surface for about
10 fifteen minutes. Immediately after removing the
support from the solution, the non-bound PEO star
molecules are rinsed off with water. The support
surface with the bound PEO stars are then contacted at
once with the intended biopolymer. The biopolymer can
15 be heparin, IgG, oligopeptides, etc. After a period of
time, which can be from about two to about sixty
minutes depending upon the biopolymer, the support
surface is rinsed to remove and recover any unbound
biopolymers. For the biopolymer to attach to the
20 support surface, one out of f arms of the star needs to
be covalently connected to the surface and another star
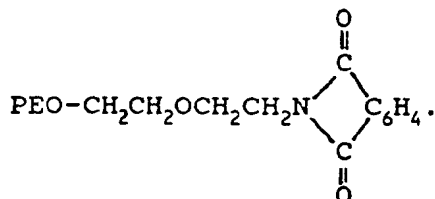arm out of f-1 needs to be covalently connected to the
biopolymer.

Alternatively, it is possible to prepare star PEO
25 molecules with a group other than a hydroxyl, such as
an amino group ($-NH_2$), or a benzyl bromide group
($-C_6H_4CH_2Br$). A PEO chain having a sodium alkoxide
($-ONa$) end group is reacted with
N-(2-bromoethyl)phthalimide:

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$$BrCH_2CH_2N \overset{\overset{\displaystyle O}{\underset{\displaystyle \|}{C}}}{\underset{\underset{\displaystyle O}{\underset{\displaystyle \|}{C}}}{}} C_6H_4$$
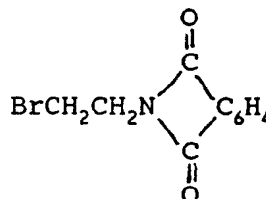
to form to a PEO phthalimide:

$$PEO-CH_2CH_2OCH_2CH_2N \overset{\overset{\displaystyle O}{\underset{\displaystyle \|}{C}}}{\underset{\underset{\displaystyle O}{\underset{\displaystyle \|}{C}}}{}} C_6H_4 .$$

The PEO phthalimide then undergoes hydrolysis by acidified water to form $PEO-CH_2CH_2OCH_2CH_2NH_2$ while releasing phthalic acid. Another method includes reacting a hydroxy terminated PEO with a dibenzyl halide, such as $BrCH_2-C_6H_4-CH_2Br$ to form a PEO chain with $PEO-CH_2CH_2OCH_2-C_6H_4-CH_2Br$. The benzyl bromide is then reacted with ammonia in methanol to create benzylamine at the end of the PEO. A PEO star molecule which is terminated by amino groups can be attached to a surface containing epoxy groups by direct reaction. Alternatively, the groups can be attached to a surface containing additional amino groups or to biopolymers having amino groups by a dialdelhyde, e.g. glutaraldehyde, followed by reduction of the imine (Schiff base) by sodium borohydride.

Due to the number of available PEO arms which can accommodate ligands, the hydrogels of this invention

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can be used to continuously separate, purify and
concentrate therapeutic proteins. Processing of the
proteins will require cycles of coupling and decoupling
of the ligate to affinity ligands bound to the stars.

5   The affinity surface can be of any geometric
shape, such as particles packed in beds, freely moving
particles and porous membranes. Silica particles can
be coated with PEO stars. In this case, polyethylene
oxide is physically adsorbed to the silica surface but

10 cannot be covalently bound unless the surface of the
silica has been previously modified. Radiation can be
used to cross-link physically adsorbed PEO star
molecules to each other and in addition to silica
surfaces which have been modified by vinyl silane.

15 Thereby, the polyethylene oxide stars forms a shell
covering the particle. The PEO star molecules can also
be deposited into pores of ultrahigh molecular weight
polyethylene such as Porex™ (Auburn, Georgia), on the
surface of Goretex™ e-PTFE (expanded

20 polytetrafluoroethylene) and Mylar™ film.

        As explained above, an immobilized layer of PEO
star molecules on a support may have been formed by
first creating reactive ends (e.g. tresyl) on the PEO
star arms, which react with a specific group in the

25 surface to form a covalent bond. This will leave
numerous other reactive ends on the bound star
molecules available to react with and bind affinity
ligands. Alternatively, the immobilized layer of PEO
star molecules may have been formed by radiation cross-

30 linking.

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In the latter case, once a PEO star molecule layer
is affixed on the support surface, the terminal
hydroxyl groups of the PEO arms are activated by
tresylation or by other reaction previously described.
5   The activated PEO star molecules in the immobilized
layer are then exposed to the affinity ligand under
conditions that favor covalent binding, for example pH
10 or above if the affinity ligand contains amino
group. Examples of preferred ligands include, but are
10 not limited to antibodies and F_{ab} fragments thereof,
Protein A, active polysaccharides, heparin-NH_2, anti-
Protein C IgG, and the F_{ab} fragment of anti-Protein C
IgG.

Following affinity bonding of a specific ligate to
15 its bound ligand, the PEO star-coated affinity support
is washed to remove unbound proteins. Remaining bound
proteins are then decoupled by changing the composition
of the eluting buffer, for example by changing the
ionic strength or the pH (e.g., to pH 10 or above) of
20 the eluting buffer. For example, a 1M NaCl decoupling
solution can be used in the case of anti-thrombin III
bound to heparin. The decoupling results in free
ligate in the eluting buffer. The ligate can then be
separated from the eluting buffer using known
25 techniques, such as by diafiltration described by Herak
et al., Biotech. Prog. 5:9-17 (1989). Separated
ligates can then be concentrated using known
techniques. Examples of some specific ligates include
but are not limited to macromolecules, monoclonal
30 antibodies, antigens, proteins, peptides, viruses and

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cells (e.g., blood platelets, white blood cells, endothelial cells and other non-blood cells).

In addition to bioseparations, the hydrogels made according to this invention are useful for a variety of
5 biomedical applications, due to their non-thrombogenic properties and excellent mechanical durability. They are suitable for _in vivo_ applications in which blood contact is required, including blood contacting implantable vascular prostheses, angioplastic stents,
10 cardiovascular sutures, metabolic support catheters, angioplastic balloon catheters, artificial hearts and ventricular assist devices. The hydrogels may also be used for _ex vivo_ devices, such as hemodialysis membranes and membranes for extra-corporeal
15 oxygenators.

A preferred application for the star molecules of this invention is in the manufacture of contact lenses. PEO star molecules can be grafted onto a suitable art recognized contact lens material, such as gas permeable
20 lenses, using the techniques described herein. For example, the contact lens material can be immersed in a PEO star molecule solution and exposed to ionizing radiation to thereby graft the star molecules onto the contact lens surface. Alternatively, the surface of
25 the contact lens materials can be modified by creating amino or thiol groups on it surface. The modified lens material is then exposed to activated PEO star molecules, such as tresylated star molecules described above. Due to the properties of the star molecules,
30 absorption of proteineous deposits from natural enzymatic secretions of the eye by the star molecule

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coated-contact lens material can be eliminated or
substantially reduced. Thus, the coated lenses will
not become clouded or opaque because of lowered protein
absorption.

5   Additional chemical components can be incorporated
into the PEO star layers depending upon the
application. In some instances it may be advantageous
to incorporate heparin to further reduce
thromogenicity. While heparin can be attached
10   covalently to tresylated hydroxyls on the star
molecules, it is also readily incorporated at high
concentrations in hydrogel layers which are formed by
simply adding it to the solution of the PEO stars
before irradiation. In this form it elutes into the
15   blood flow over a significant period of time.
           The invention will be further illustrated by the
following non-limiting Examples:

Example 1

Synthesis and Characterization of Various PEO Hydrogels
20   Linear PEO and various forms of star molecules
having the physical properties described below were
electron beam irradiated, at a dose rate of about 0.1
megarads per second, and with a two megarad dose per
pass under the beam to form unsupported hydrogels, in
25   order better to study swelling phenomena. However, in
practice the PEO solutions would be simultaneously
cross-linked and covalently bonded to support surfaces
by radiation. Radiation was delivered from a 3 MeV Van

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de Graaff generator (MIT High Voltage Research
Laboratory).

Table 2 presents the apparent swelling ratio q at
25°C (q = volume of hydrogel equilibrated in
5   water/volume of original mixture irradiated) as a
function of radiation dose D in megarad, and as a
function of the star type. Two liner PEO samples are
included for reference. The concentration of the
solution as irradiated in every case was 10.0 wt/vol.%
10 in MilliQ® water (Millipore Corp., Bedford, MA). From
Table 2 it is apparent that the swelling ratio q of
hydrogels formed from star molecules is significantly
less than for hydrogels from linear PEO types.
Furthermore, the high styrene content Type II hydrogels
15 (3103, 3229) exhibit virtually no swelling.

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TABLE 2

Swelling Ratios q of 10 wt/vol.% Polymer/Water After Electron Beam Irradiation

Linear PEO		D	q	[OH] μM
Nominal 300,000 M.W.		4	2.03	0.33
		6	1.92	0.35
Nominal 100,000 M.W.		4	2.8	0.71
		6	2.4	0.83

Type I Stars (no styrene)

	M.W. Total	# Arms	M_{PEO}	D	q	[OH] μM
3098	229,000	43	5,300	4	1.3	14.6
3210	142,000	40	3,460	4	1.4	20.0
3224	79,000	8	10,000	6	1.6	6.3

TABLE 2 cont'd

Type II Stars

	M.W. Total	%S	# Arms	M_{PEO}	M_{PS}	D	q	[OH] μM
3103	190,000	20	16	8,000	2,000	4	~1.0	8.4
3229	257,000	30	25	6,800	3,200	4	~1.0	9.6
3385	371,000	2	30	12,000	520	4	1.7	4.7
						6	1.6	5.7

D: dose in megarads.
q: Swelling Ratio.
[OH]: gram equivalent per liter of gel swollen to
    equilibrium in water at 25°C.
Total M.W. of stars by light scattering.

From the results, the random cross-linking of star molecules cannot be expected to lead to networks like

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those produced from randomly cross-linked linear
macromolecules, in which the functionality of the
junction ϕ is necessarily four. In contrast, the
incorporation of stars implies incorporation of
5   junctions of high functionality ϕ, i.e., ϕ = number of
arms. Further, the "junction" is in effect a high
modulus poly DVB core, in Type I stars, and an even
more complicated entity, i.e., poly DVB with short
polystyrene arms, in Type II stars. Thus, the space
10 occupied by the "junction", and the thermodynamically
adverse junction-water interaction place the star
hydrogel beyond the tenets of the Flory-Huggins theory
of swelling of randomly cross-linked networks.

    The last column in Table 2 shows the molar
15 hydroxyl content of the gel at equilibrium in water
[OH], calculated as: (mols OH/100 g. dry polymer)q^{-1},
wherein the first term is determined as (number of
arms/total M.W.)·100. Each original solution at 10
wt/vol.% contains 100 grams of dry polymer per liter.
20 The final wt/vol.% polymer in the gel at equilibrium
with water is thus 10/q. This is very important if the
star hydrogel is to be deployed as a model biomaterial
to which bioactive species are to be grafted. It is
desirable to have a high value of [OH] and a low
25 swelling ratio q in order that the biomaterial remain
approximately in the shape in which it was cast. This
is especially important for the case that the hydrogel
has been formed on a support which is incapable of
swelling or shrinking. Stars 3098 and 3210 as
30 hydrogels provide examples.

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In the hydrated state, i.e., in equilibrium with
blood plasma, preliminary studies of platelet
deposition indicate that the surface of star hydrogel
is entirely PEO, that is, the poly DVB core is buried
5   and inaccessible, because of the fact that the star
hydrogel acts as if it were a hydrogel of linear PEO.
Cross-linking of these arms is random, granting that
all PEO arms have approximately the same molecular
weight on a given star type as a consequence of the
10   anionic polymerization route. Under an electron beam,
hydroxyl radicals created from water constitute the
principal reagent and therefore, the PEO rather than
the poly DVB and PS experiences macroradical formation
and subsequent coupling. To some degree scission of
15   the arms must occur competitively with cross-linking
under radiation. The terminal hydroxyl concentrations
[OH] calculated in Table 2 do not take this into
account.

Biocompatibility
20   Hydrogels containing Type I Stars 3098 (no
polystyrene content) or Type II Stars 3385 (2%
polystyrene, 98% PEO), described above, were examined
for biocompatibility.
        Tubular specimens of hydrogel were prepared from
25   10 wt/vol.% solutions of star polymers 3098 and 3385
using 0.7 ml of solution centrifugally cast and
irradiated under six megarads inside glass tubes of 10
cm length x 9 mm lumen. These were tested in an _ex_
vivo shunt model (indium 111 labeled platelets, baboon)
30   with uncoated glass tubes as control. Over a period of

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one hour at a blood flow rate of 100 ml/min., there was
no increase of indium count above background for the
two hydrogel surfaces, whereas in glass control tubes
(no coating) the count more than trebled over
5 background.

Using similar techniques, glass tubes lined with
0.7 ml hydrogels formed from 10 wt./vol. % solutions of
linear PEO of 100,000 and 300,000 M.W., respectively,
under the same does were prepared. Upon equilibration
10 at 25°C with pure water, the apparent swelling ratios
(final volume:initial volume) were 1.3, 1.3, 2.8 and
2.0 for Star 3098, Star 3385, PEO 100,000 and PEO
300,000 hydrogels, respectively. Values of 1.3, as
compared to two or more, mean that the star polymer
15 based hydrogels when exposed to blood do not expand to
such a degree as to compromise attachment to the
surface on which they were cast. The lack of platelet
uptake indicates that the star polymers in hydrogel
form present a "pure" PEO surface to blood. As such,
20 the DVB cores were shielded from access of plasma
proteins by the PEO arms.

Example 2

Synthesis of PEO Star Molecule Coated Contact Lenses
A series of gas permeable contact lenses were
25 coated with PEO star molecules. The lenses were type
RXD manufactured by Polymer Technology Corporation and
were ground to the correct shape from cylindrical
wafers which were cut from rods. The rods were made by
polymerization of perfluoroacrylates and methacrylates,

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and had high oxygen permeability. The lenses were
considered to be rigid.

The convex and then the concave side of the lenses
were exposed to Plasma Enhanced Chemical Vapor
5 Deposition (PECVD) in a Plasmatherm® Dual Chamber
device, under the following conditions: five minute
time exposure, 100 watts at 13.56 MHz and 104 DC volts.
Ammonia gas was flowed through the chamber at 20
standard cubic centimeters per minute at a pressure of
10 500 millitorr and a temperature of 30°C.

Following this treatment, the lenses were analyzed
by X-ray Photoelectron Spectroscopy (XPS), also called
ESCA. The control lenses had zero atom percent
nitrogen, and the treated lenses had an average of
15 about five atomic percent nitrogen which was presumably
in the form of amino groups.

PEO star molecule 3509 (number assigned by P.
Rempp) had the following characteristics as reported by
Rempp:

20   1.   Weight average molecular weight M_w: 576,000;
      2.   Molecular weight of PEO arm as calculated
            from ratio of ethylene oxide to potassium
            naphthenide. M_{arm} = 10,000; and
      3.   Deduced number of arms per star f_w = 57.6,
25   thus the weight average number of hydroxyls
            per molecule is also 57.6.

Following the procedure recommended by Nilsson et
al., Methods in Enzymology, Vol. 104, p 55 (Academic
Press 1984), 1.0 gram of the star material was

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dissolved in 15 grams dichloromethane (dry) at -10°C.
100 µl triethyl amine and then 73 µl trifluoroethane
sulfonyl ("tresyl") chloride ($CF_3CH_2SO_2Cl$) were added to
the dichloromethane solution. The dichloromethane and
5 triethylamine had been dehydrated by storage over
molecular sieves.

The reaction was allowed to proceed for 90 minutes
as the temperature rose from -10°C to +20°C. The
polymer was then precipitated when cold anhydrous
10 methanol was added. The precipitate was centrifuged to
form a pellet, rewashed twice with cold methanol, and
then dried under vacuum at 25°C. Elemental analysis
for sulfur showed greater than 80% tresylation of the
original hydroxyls.

15 The ammonia plasma treated lenses (APTL lenses)
were coated by the activated star molecules which were
accomplished by contacting each lens with 1 ml of a
tris buffer (pH 10) containing 100 mg of tresylated
stars (PEO 3509) at room temperature. The stars
20 remained in the solution for 60 minutes.

The lenses were then repeatedly rinsed with
MilliQ® water. Following drying, the lenses were
analyzed again by XPS. Significant nitrogen was found
in the surface, ca. 2.5 atomic percent. This is
25 consistent with the presumed thickness of the attached
PEO star layer. It was determined by intrinsic
viscosity that the average PEO star molecule has a
diameter (as an "Einstein" model sphere) of 250
angstroms (25 nm). With the assumption that each star
30 molecule occupies a circular area of the same diameter,
the removal of water from this "tethered" spherical

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molecule will result in its collapse to a thickness on
the order of 12 angstroms (1.2 nm) which was
sufficiently thin to allow XPS radiation to probe the
contact lens substance under this layer. The coating
5   had in fact resulted which was proved by attempting
rewetting with water of ammonia plasma treated lenses
(APTL). The same occurred after exposure to the
tresylated PEO stars.

The APTL lenses permitted the water to "bead up,"
10   i.e., water did not form a continuous layer, whereas,
by contrast the APTL with PEO attached allowed water to
form a continuous film on both convex and concave
surfaces. Several drying and rewetting steps were
carried out with the same result, indicating that the
15   PEO stars were covalently attached.

<u>Equivalents</u>

Those skilled in the art will recognize, or be
able to ascertain, using no more than routine
experimentation, many equivalents to the specific
20   embodiments of the invention described herein. Such
equivalents are intended to be encompassed by the
following Claims:

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CLAIMS

1. A method of immobilizing polyethylene oxide star
    molecules to a support surface to form a layer
    thereon, comprising the steps of:
    a)   exposing an organic solution comprising
          polyethylene oxide star molecules each of
          which consists essentially of a plurality of
          hydroxy-terminated polyethylene oxide chains
          attached to a divinyl benzene core to a
          reagent to affix reagent groups to the
          hydroxy termini, said reagent groups
          permitting subsequent attachment of amino or
          thiol groups to the PEO chains ends by
          displacement;
    b)   separating the polyethylene oxide star
          molecules with active reagent end groups from
          the organic solvent;
    c)   dissolving the activated polyethylene oxide
          star molecules in an aqueous solution at an
          appropriate pH; and
    d)   contacting the solution of step (c) with a
          support surface containing amino and/or thiol
          groups to covalently bind the reagent
          terminated star molecules, thereby
          immobilizing the reagent terminated star
          molecules in a dense layer to the support
          surface.

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2. A method of immobilizing polyethylene oxide star
    molecules to a support surface to form a layer
    thereon, comprising the steps of:
    a) providing an aqueous solution comprising
        polyethylene oxide star molecules each of
        which consists essentially of a plurality of
        hydroxy-terminated polyethylene oxide chains
        attached to a divinyl benzene core, the star
        molecules being essentially non-adsorptive
        toward biomolecules and suitable for use
        wherein blood contact is necessary;
    b) depositing the aqueous solution onto a
        support surface; and
    c) irradiating the solution with electron
        radiation to sufficiently cross-link the
        polyethylene oxide chains to each other and
        to the support to produce a layer of cross-
        linked star molecules which is thereby
        immobilized to the support surface.

3. A method of immobilizing polyethylene oxide star
    molecules to a support surface to form a layer
    thereon, comprising the steps of:
    a) providing a solution comprising polyethylene
        oxide star molecules each of which consists
        essentially of a plurality of hydroxy-
        terminated polyethylene oxide chains attached
        to a polymeric core, the star molecules being
        essentially non-adsorptive toward
        biomolecules and suitable for use wherein
        blood contact is necessary;

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b)   depositing the solution onto a support
      surface; and
c)   reacting the solution sufficiently to produce
      a PEO star layer which is immobilized to the
      support surface.

4.   The method of Claim 3, wherein step (c) is
      performed by irradiating (e.g., electron beam
      radiation) the solution to produce a layer of
      cross-linked star molecules; wherein the solution
      is an aqueous solution.

5.   The method of Claim 3, further comprising the
      steps of first reacting the hydroxyl ends of PEO
      star molecules with tresyl chloride to form tresyl
      functions, then transferring the tresylated star
      molecules into an aqueous solution at a pH of
      above about ten prior to step (b); and wherein the
      support surface contains active functional groups
      (e.g., thiol, amino or both) for immobilizing the
      tresylated star molecules thereto.

6.   The method of any one of Claims 1-5, wherein the
      support surface is selected from the group
      consisting of particles, porous polymeric
      membranes, polymeric film and biomedical devices.

7.   The method of any one of Claims 1-5, wherein the
      support surface is selected from the group
      consisting of blood contacting vascular
      prostheses, angioplastic stents, cardiovascular

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suture, metabolic support catheters, angioplastic
balloon catheters, artificial hearts, ventricular
assist devices, hemodialysis membranes and
membranes for extracorporeal oxygenators.

8.   The method of Claim 1, wherein the reagent is
     selected from the group consisting of tresyl
     chloride, tosyl chloride, mesyl chloride,
     epichlorhydrin, cyanuric chloride, carbonyl
     diimidazole and a mixture of succinic anhydride
     and succinimide.

9.   The method of Claim 1, further comprising the
     steps of:
     e)   washing the support surface to remove any
          non-bound star molecules, leaving the
          activated polyethylene oxide star molecules
          remaining bound thereto; and
     f)   contacting the support surface after step (e)
          with an affinity ligand of interest having
          amino and/or thiol groups thereon, to
          covalently bind the ligand sufficiently to
          the activated polyethylene oxide chains.

10. The method of Claim 9, wherein the affinity ligand
     is selected from the group consisting of
     antibodies, Protein A, F_{ab} fragments of antibodies
     and active polysaccharides (e.g., heparin).

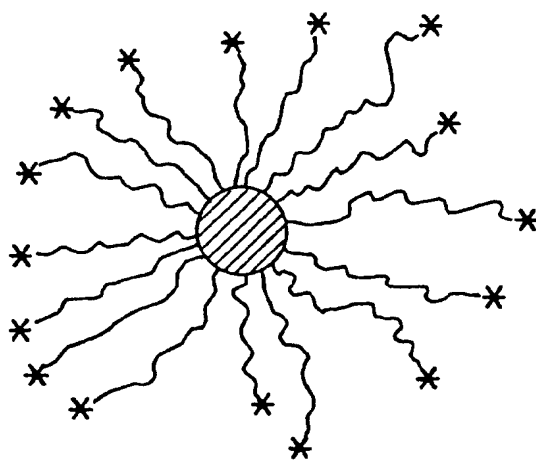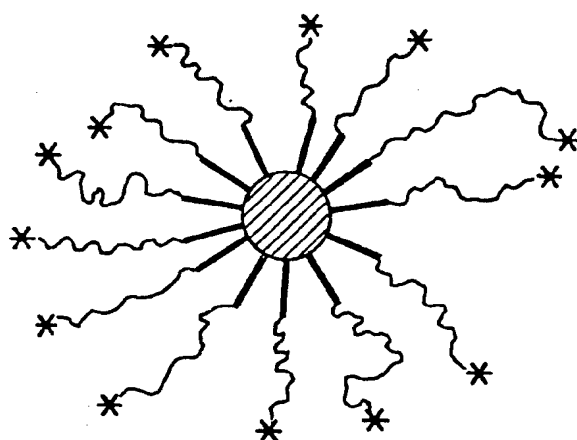11. A product produced by any one of the methods of
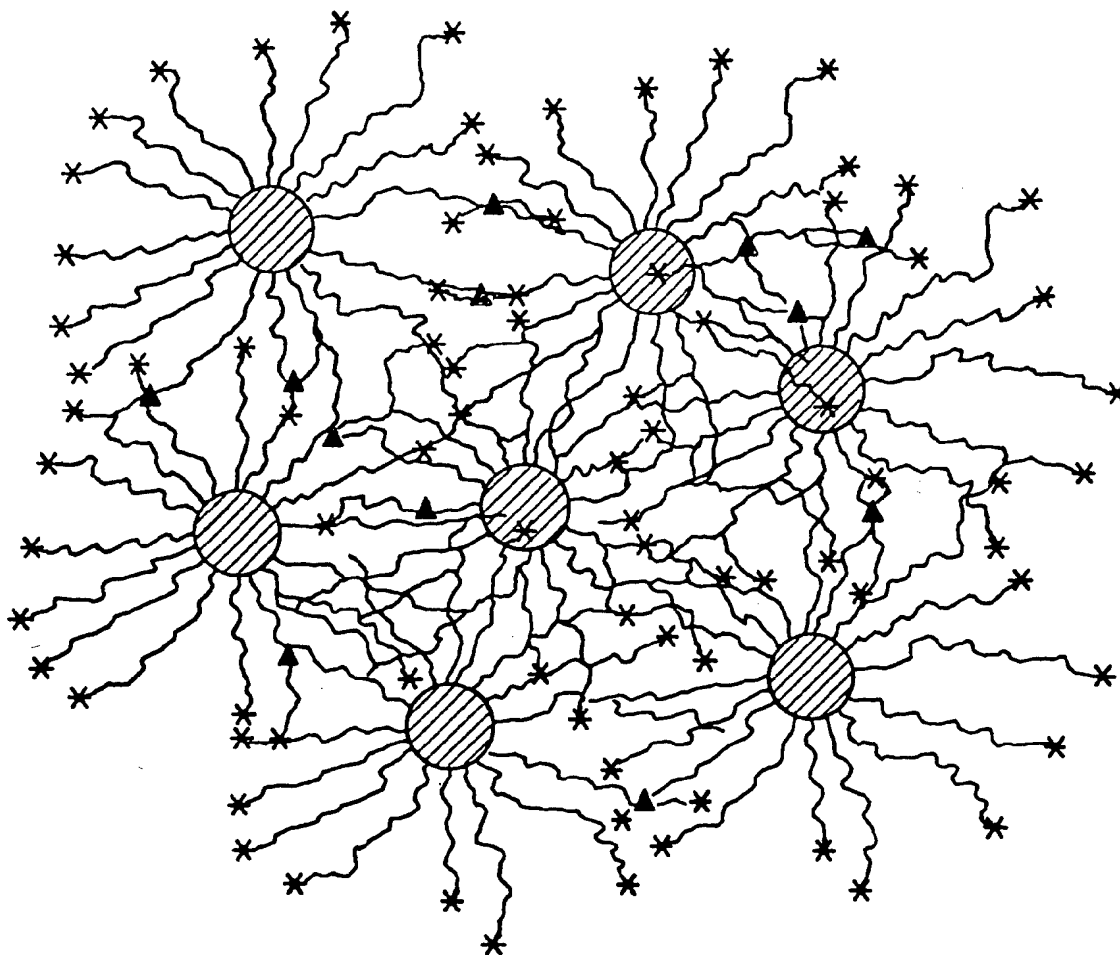   Claims 1-8.

FIG. IA

FIG. IB
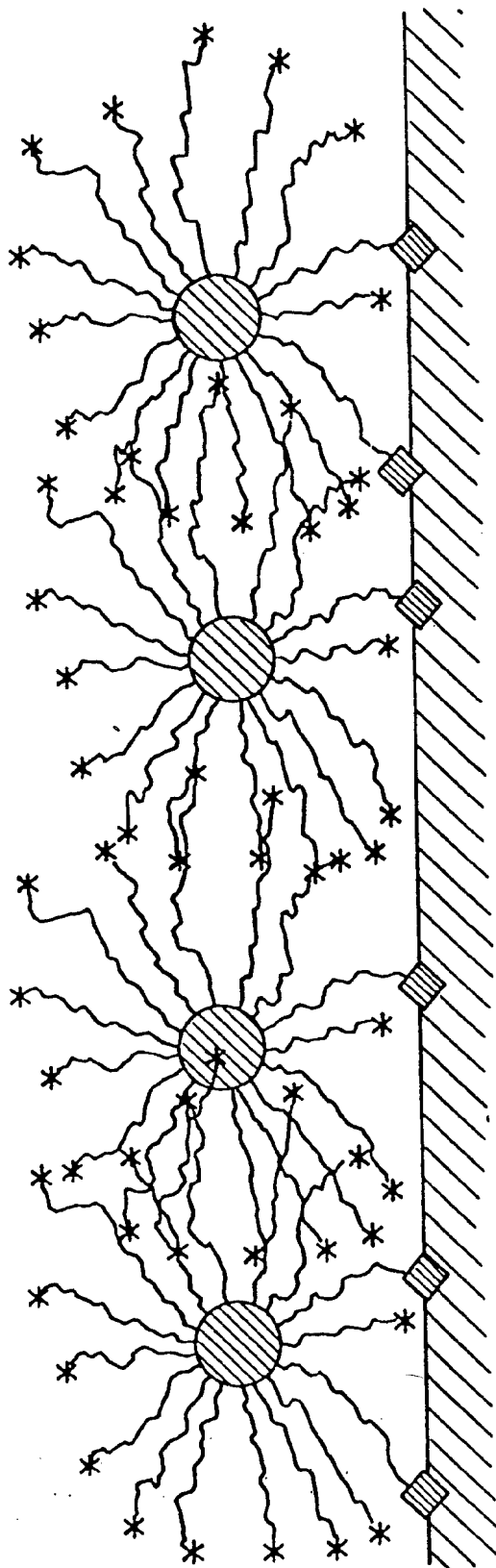
FIG. 2

FIG. 3

FIG. 4

SUPPORT SURFACE

I. CLASSIFICATION OF SUBJECT MATTER   (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61L33/00;   B01J20/32

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61L ; B01J

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[o]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	WO,A,9 112 886 (MASSACHUSETTS INSTITUT OF TECHNOLOGIE) 5 September 1991 see page 4, line 17 - page 13, line 4 see page 18 - page 21; claims 1-16; figures 1-4 ---	1-11
A	WO,A,8 602 087 (YTKEMISTA INSTITUTET) 10 April 1986 see page 11, line 20 - page 15, line 25 ---	1,2
A	EP,A,0 068 509 (TORAY IND.) 5 January 1983 see page 8, line 14 - page 9, line 8 ---	3
A	EP,A,0 332 261 (STICHTING VOOR DE TECHN. WETENSCHAPPEN) 13 September 1989 ---	
	-/--	

[o] Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 OCTOBER 1993	2 9. 10. 93

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	WENDLING J.P.

III. DOCUMENTS CONSIDERED TO BE RELEVANT	(CONTINUED FROM THE SECOND SHEET)	
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
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